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NanoLC-FTMS Based Mapping of Protein Oxidation Sites Using Element-Coded Affinity Mass Tags

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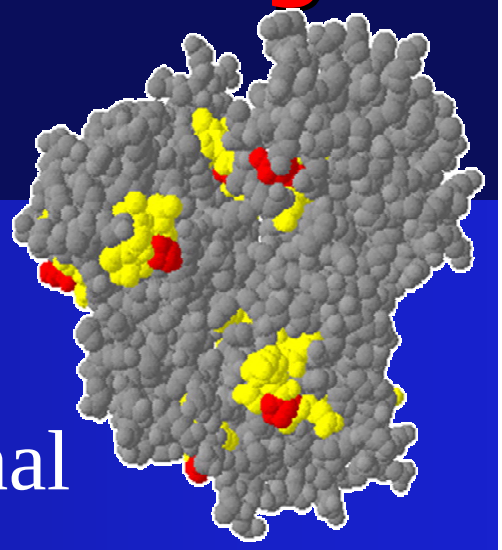
NanoLC-FTMS Based Mapping of Protein Oxidation Sites Using Element-Coded Affinity Mass Tags



Nicolas L. Young

Lawrence Livermore National Laboratory

Outline

- Protein Oxidation Background
- Analytical Approach & Instrumentation
- Identification of Protein Oxidation Sites in Serum
- Site Specific Quantitation
- Future Work

Protein Oxidation is Associated with Disease

Carbonyl end-products of protein oxidation

Have been correlated with:

Alzheimer's Disease
Cataractogenesis
Progeria
Werner's syndrome
Amyotrophic lateral sclerosis
Respiratory distress syndrome
Aging

Are thought to be associated with:

Atheroclerosis
Diabetes
Parkinson's disease
Essential hypertension
Cystic fibrosis
Ulcerative colitis

J. Biol. Chem 272, pp. 20313-20316

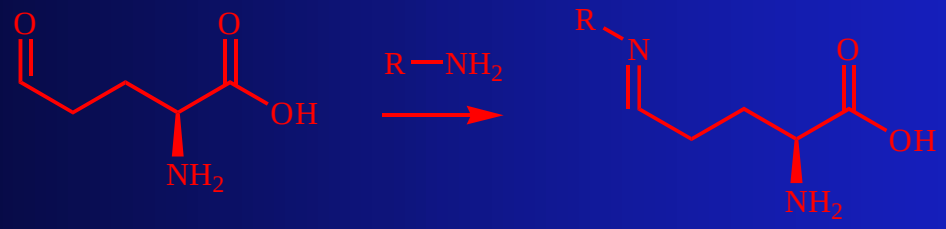
Mechanism of Oxidation

Metal ions bound in protein metal binding pockets, or free metals, catalyze the production of hydroxyl radicals which then damage surrounding amino acids, resulting in reactive aldehyde end products.

Arginine

Glutamic Semialdhyde

The reactive aldehyde may cross-link proteins by forming a Schiff base with amines leading to aggregation.



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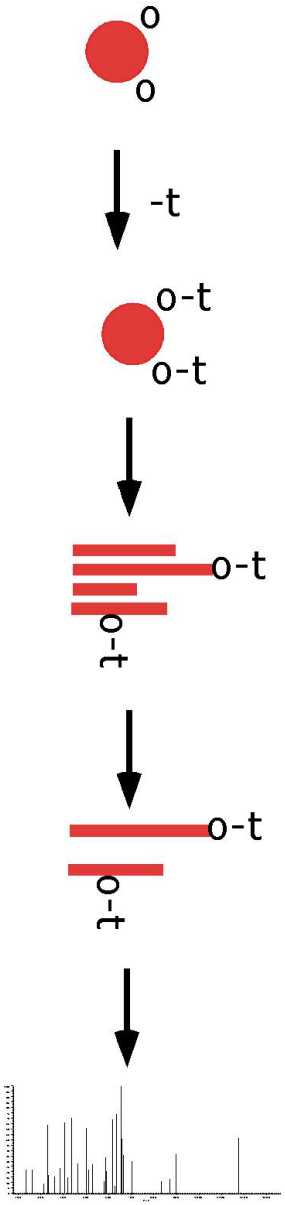
Scheme to Identify & Quantitate Sites of Oxidation

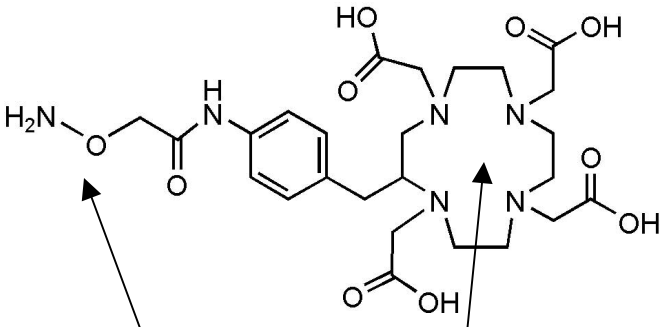


(1) Add ECAT Oxidation Tag

(2) Trypsin Digest

(3) Affinity Purify w/2D12.5 Column

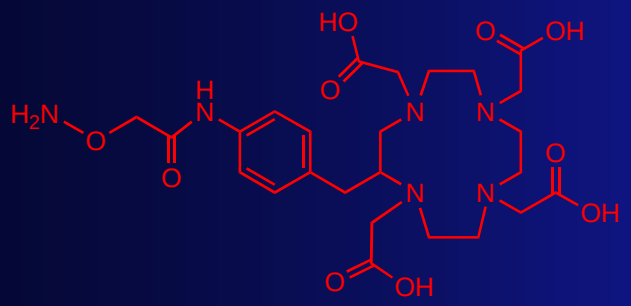
(4) Sequence with MS



Lanthanide Chelation Site

Reactive End

ECAT Allows small or large Δm and potentially large mass defect differences and multiplexing

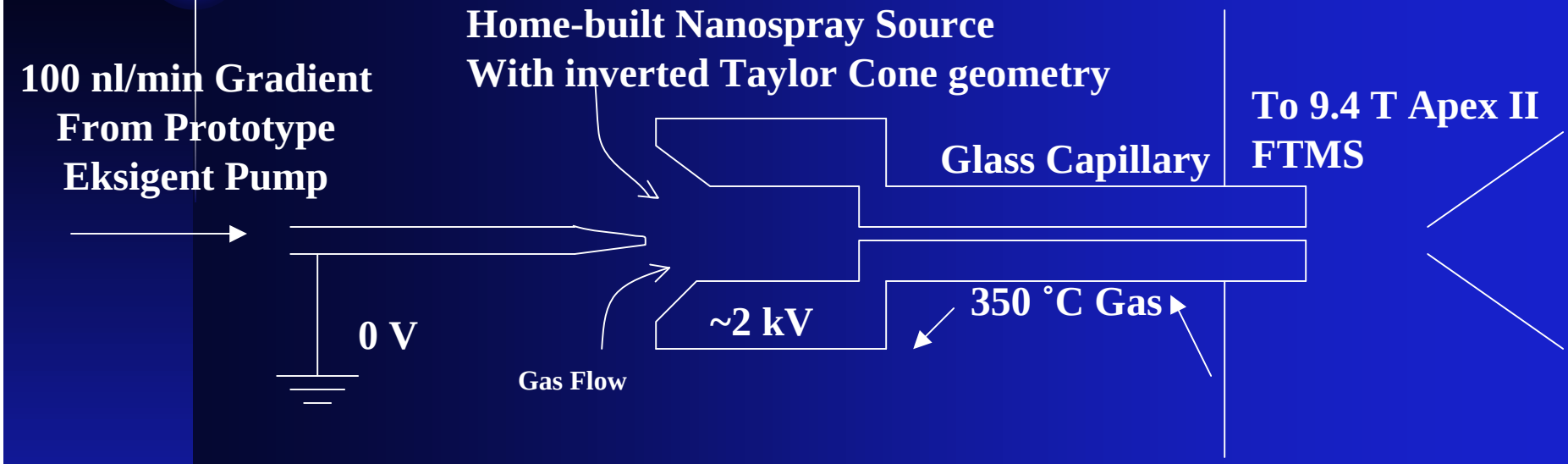


AOD - aminooxyaminobenzylDOTA

Isotope	Mass	%
^{89}Y	88.905856	100
^{139}La	138.906355	99.91
^{141}Pr	140.907657	100
^{159}Tb	158.92535	100
^{165}Ho	164.930332	100
^{169}Tm	168.934225	100
^{175}Lu	174.940785	97.4

Element	^{139}La	^{141}Pr	^{159}Tb	^{165}Ho	^{169}Tm	^{175}Lu
89Y	50.000499	52.001801	70.019494	76.024476	80.028369	86.034929
139La		2.001302	20.018995	26.023977	30.02787	36.03443
141Pr			18.017693	24.022675	**28.026568**	34.033128
159Tb				**6.004982**	**10.008875**	16.015435
165Ho					4.003893	10.010453
169Tm						6.00656

Instrumentation - NanoLC-FTICR MS

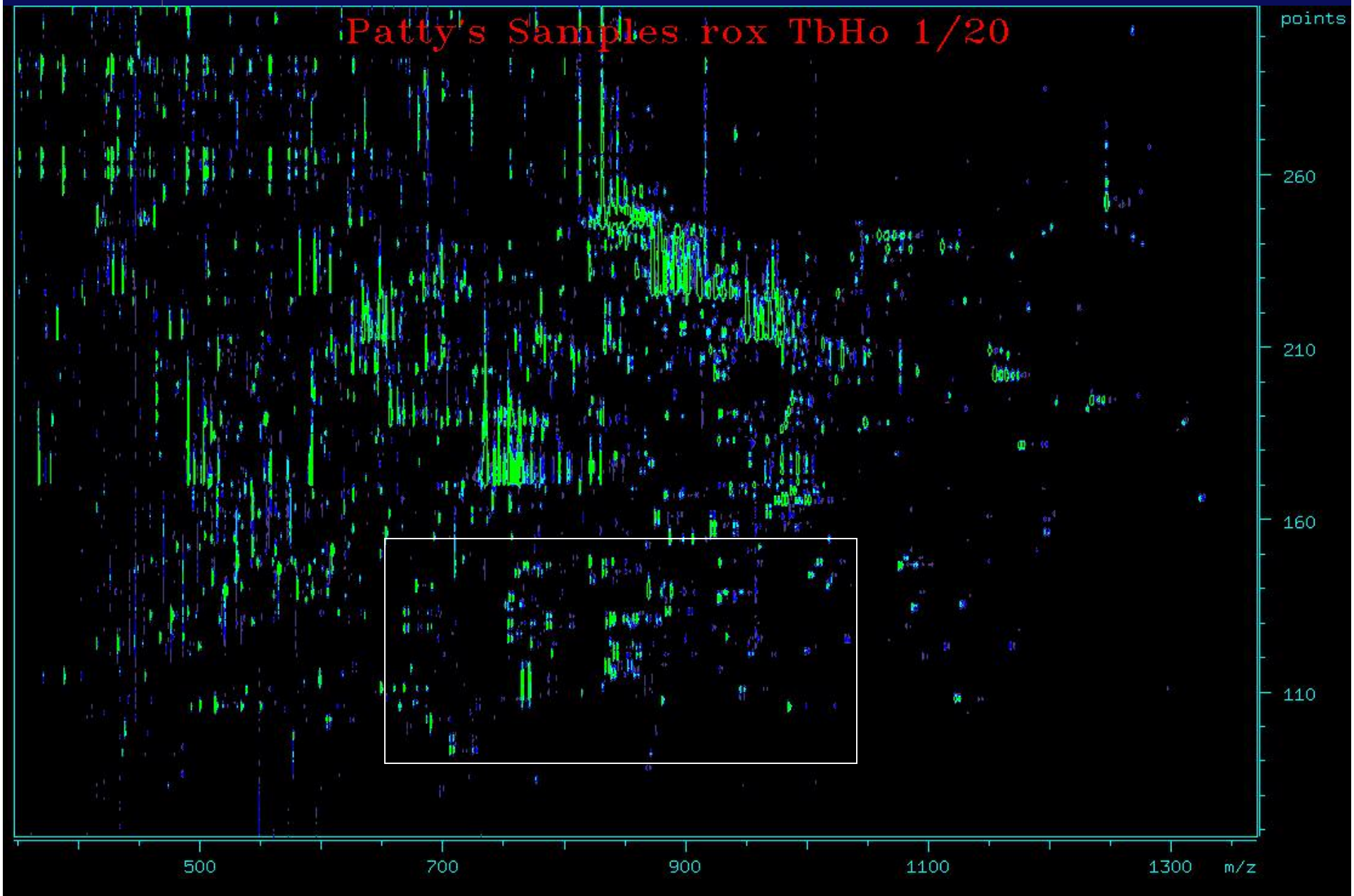


- Grounded Liquid Junction
- 75 µm I.D. Column incorporated in uncoated laser-pulled tip
- On-Axis Geometry
- Stable & Robust throughout gradient
- Sensitive spray ~ 100 zmol on column LLOD

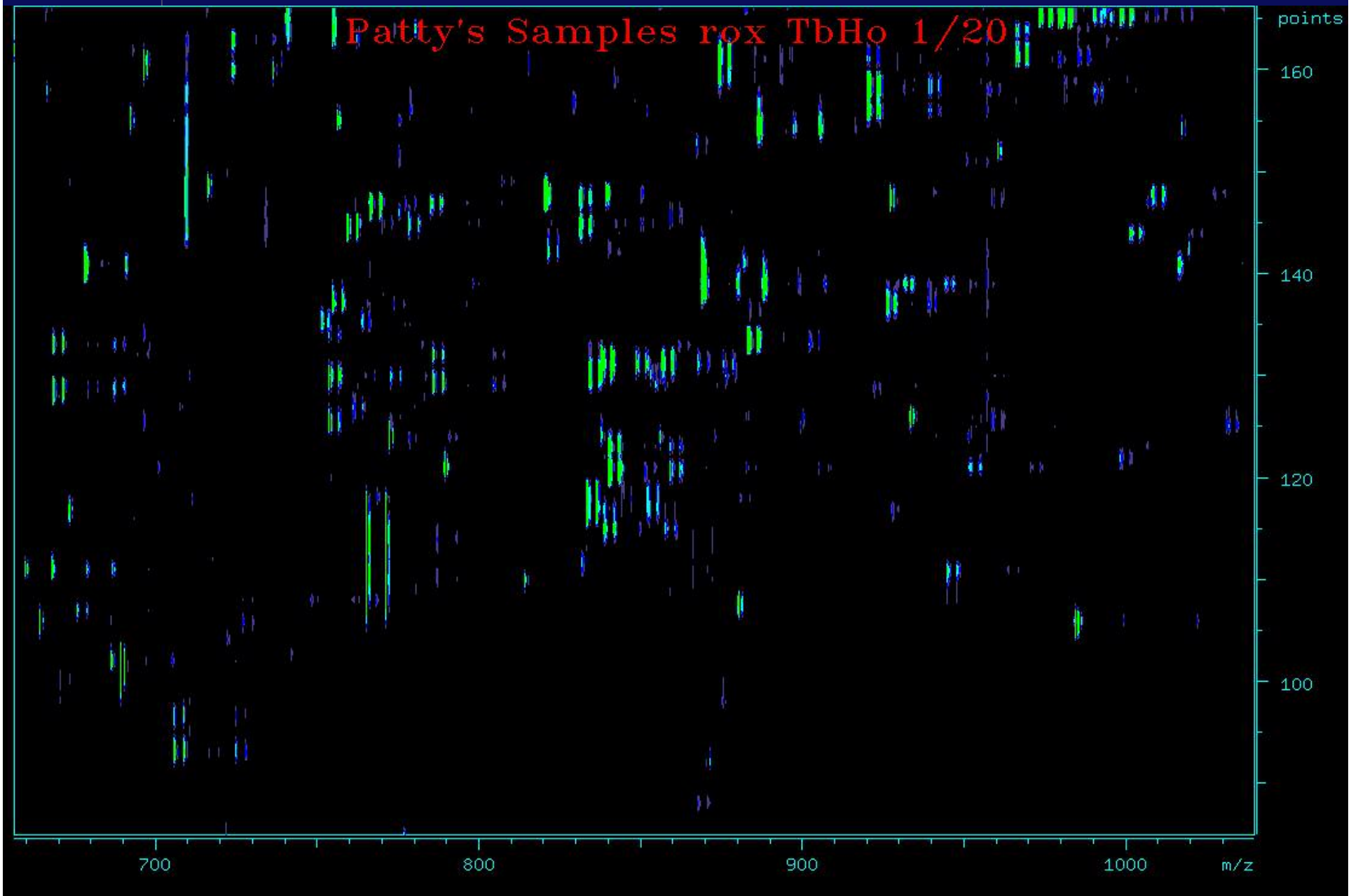
Bruker Apex II 9.4T FTMS with Modified Apollo Source

• Eight transients co-added every 0.1 minutes yielding 2D LCMS data sets

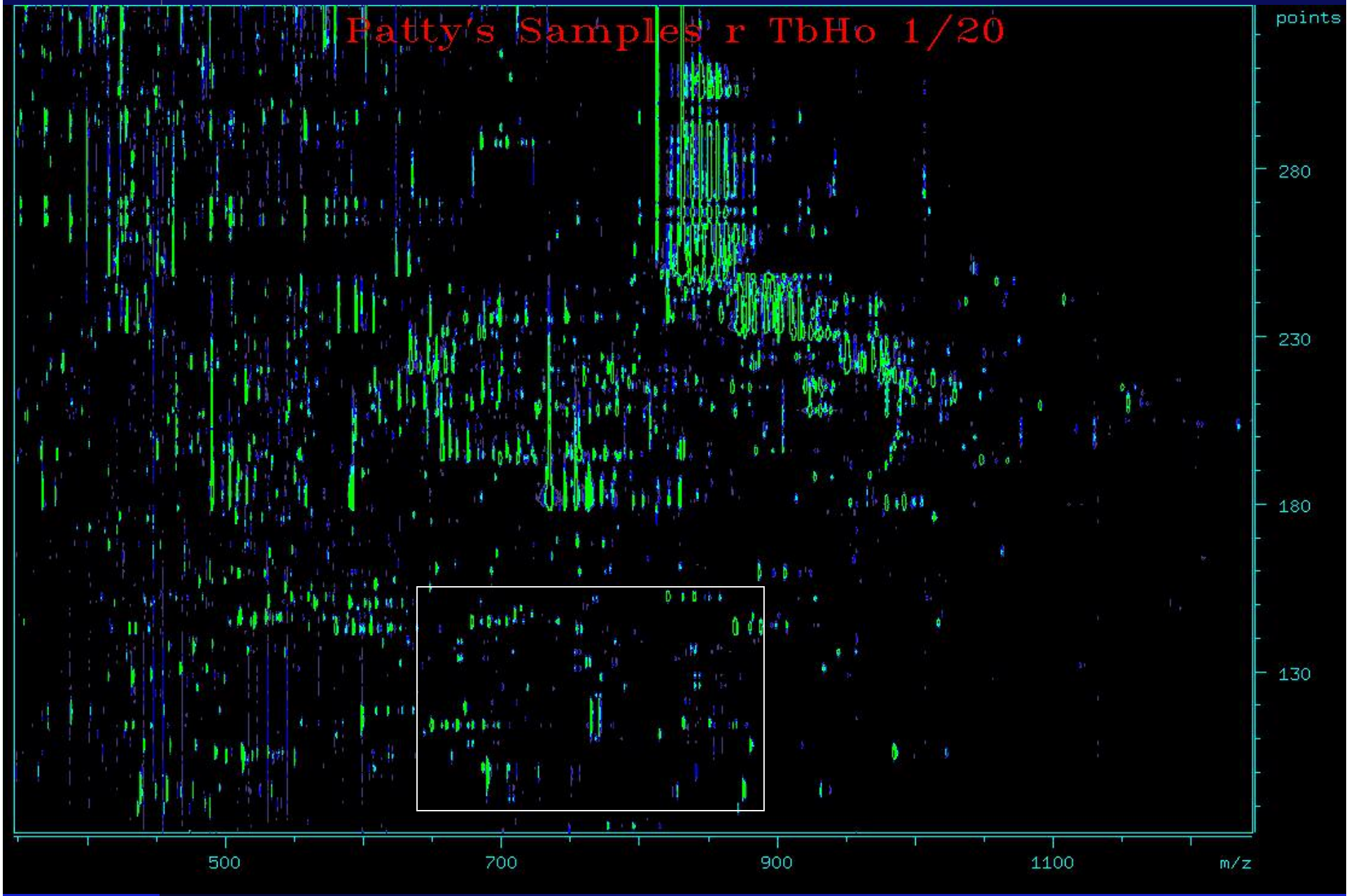
LC-FTMS of Oxidized, Labeled and Affinity Purified HSA Digest



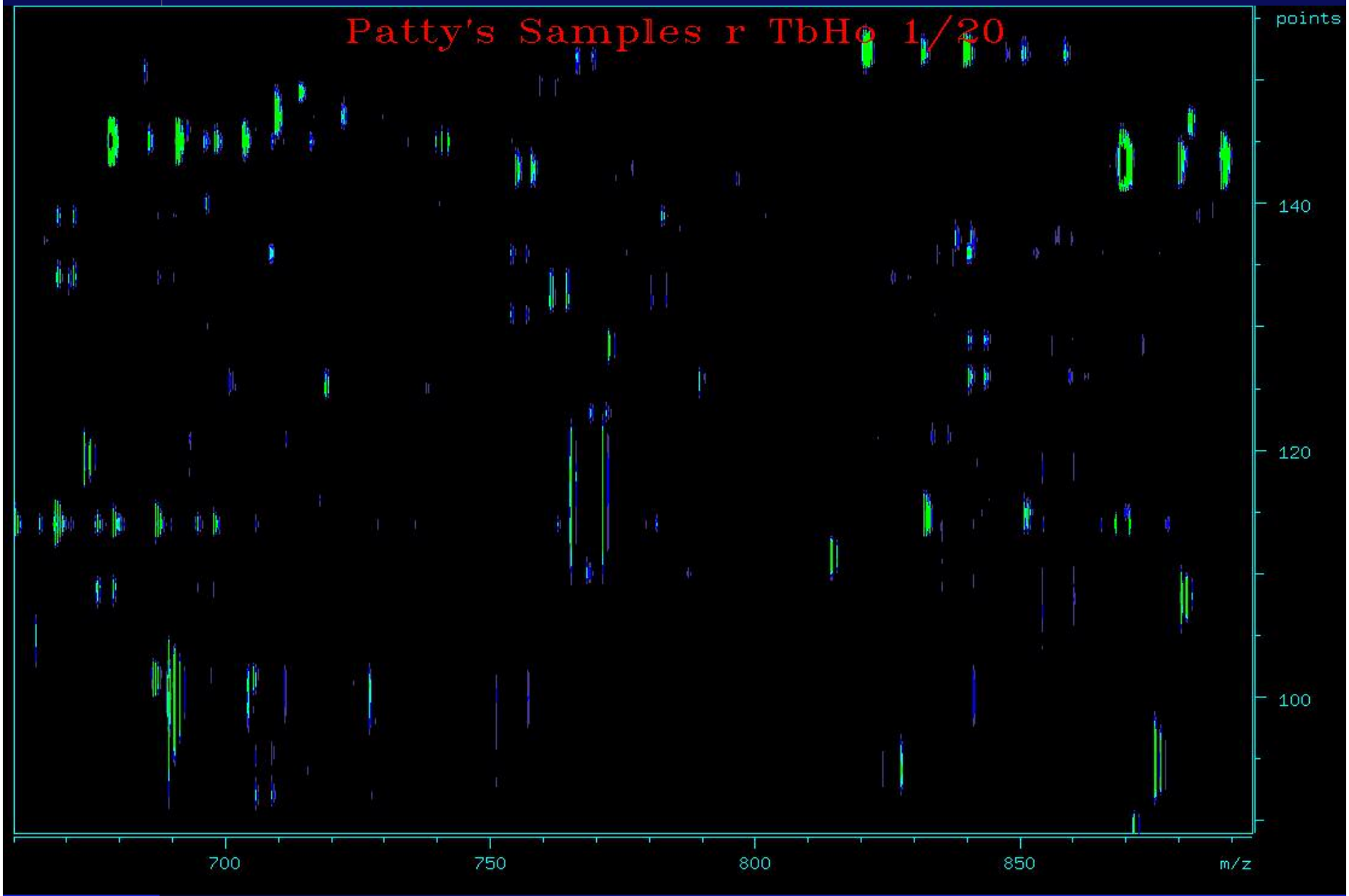
LC-FTMS of Oxidized, Labeled and Affinity Purified HSA Digest

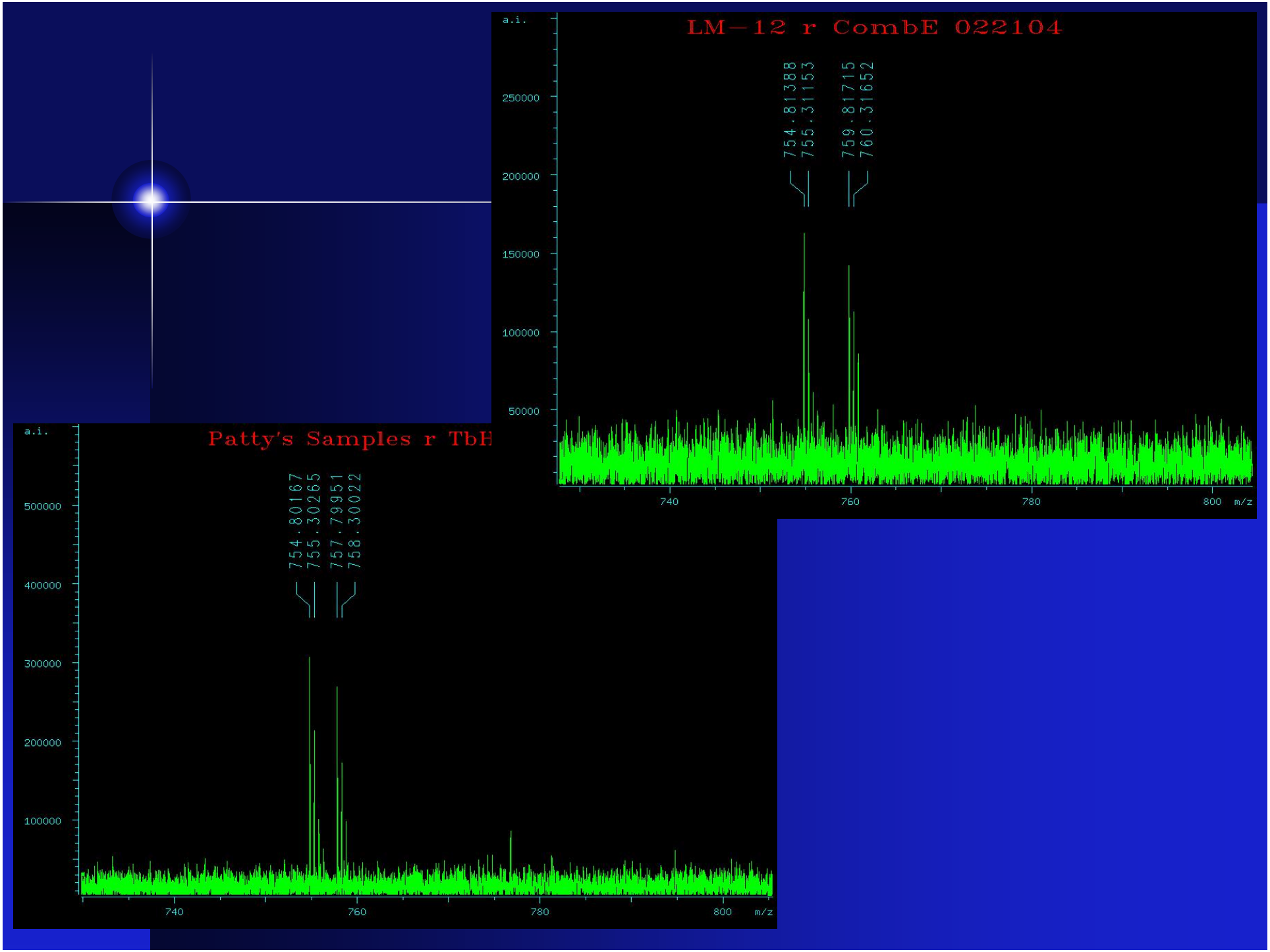


LC-FTMS of Control - Labeled and Affinity Purified HSA Digest



LC-FTMS of Control - Labeled and Affinity Purified HSA Digest




LM−12 r CombE 022104
a.i.
754.81388
755.31153
759.81715
760.31652
250000
200000
150000
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50000
740
760
780
800
m/z
Patty's Samples r TbH
a.i.
754.80167
755.30265
757.79951
758.30022
500000
400000
300000
200000
100000
740
760
780
800
m/z

Identified Peptides

rHSA Control

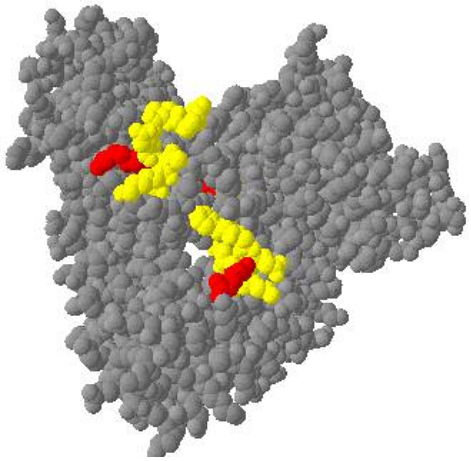
Sequence	Position
AACLLP*K	175-181
LDELRDEGK*ASSAK	182-195
FGER*AFK	206-212
TPVSDR*VTK	467-475

rHSA Oxidized

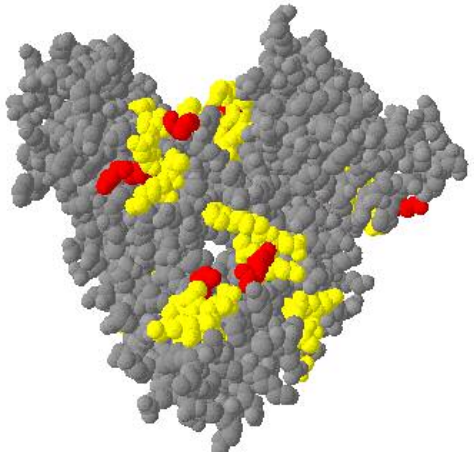
Sequence	Position	Present in Control?
FK*DLGEENFK	11-20	
DDNP*NLPR	107-114	
AACLLP*K	175-181	Y
FGER*AFK	206-212	Y
FPK*AEFAEVSK	223-233	
LAK*TYETTLEK	349-359	
CCKHP*EAK	437-444	
TP*VSDR	467-472	
TPVSDR*VTK	467-475	Y

Pre-existing vs FeEDTA/Asc Oxidized

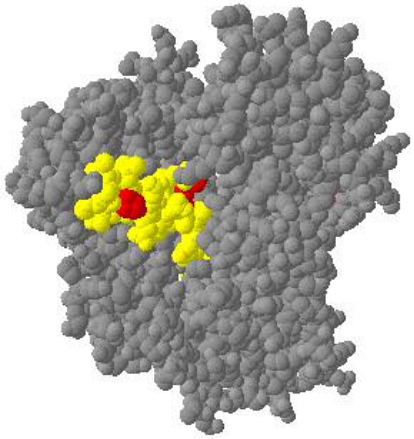
Control Front View



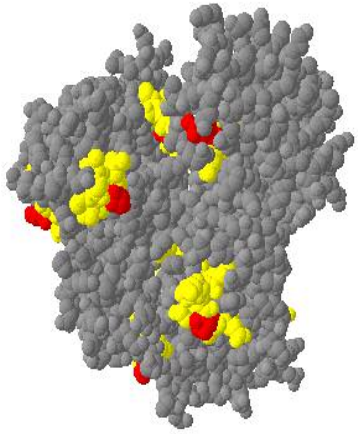
Oxidized Front View



Control Rear View



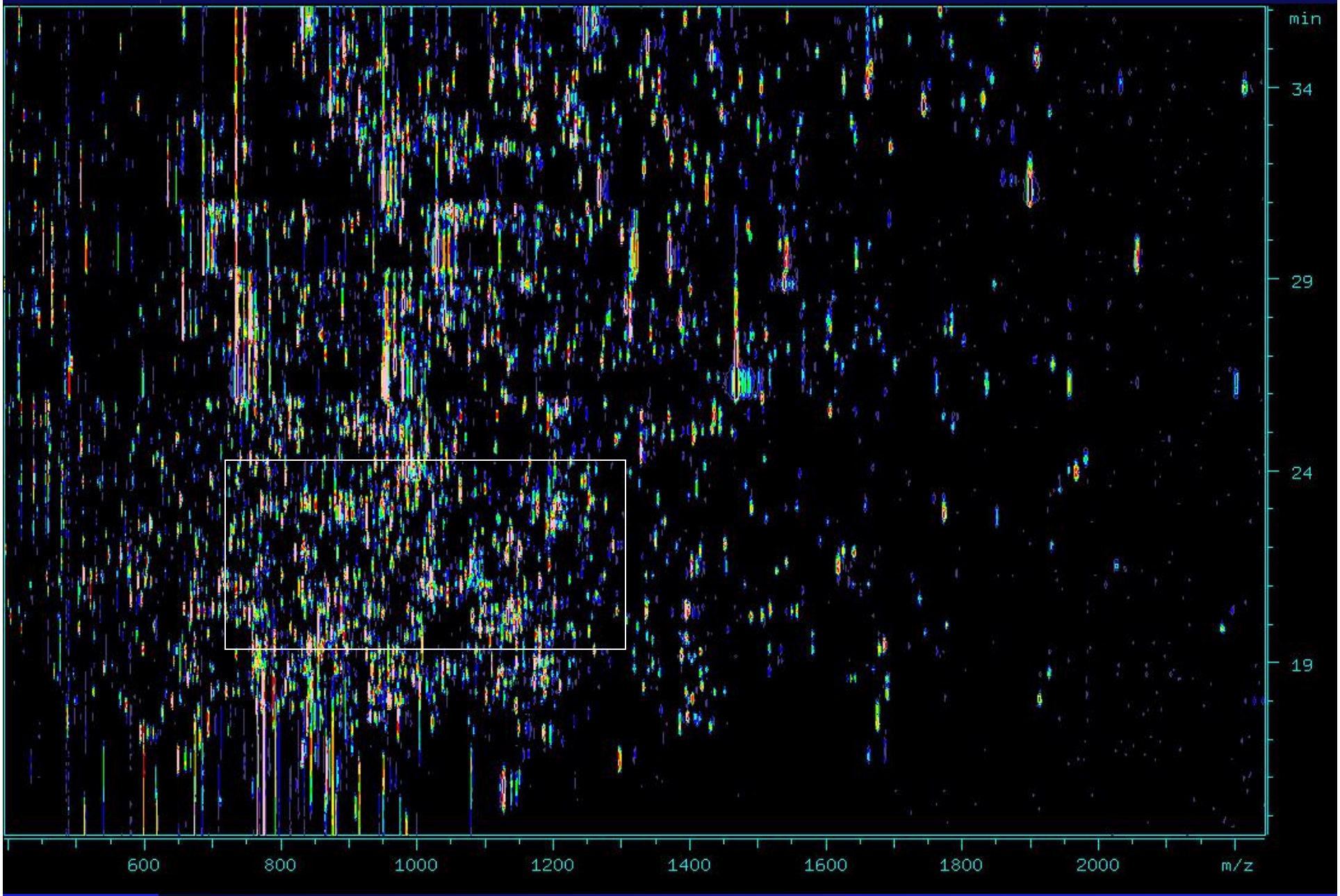
Oxidized Rear View



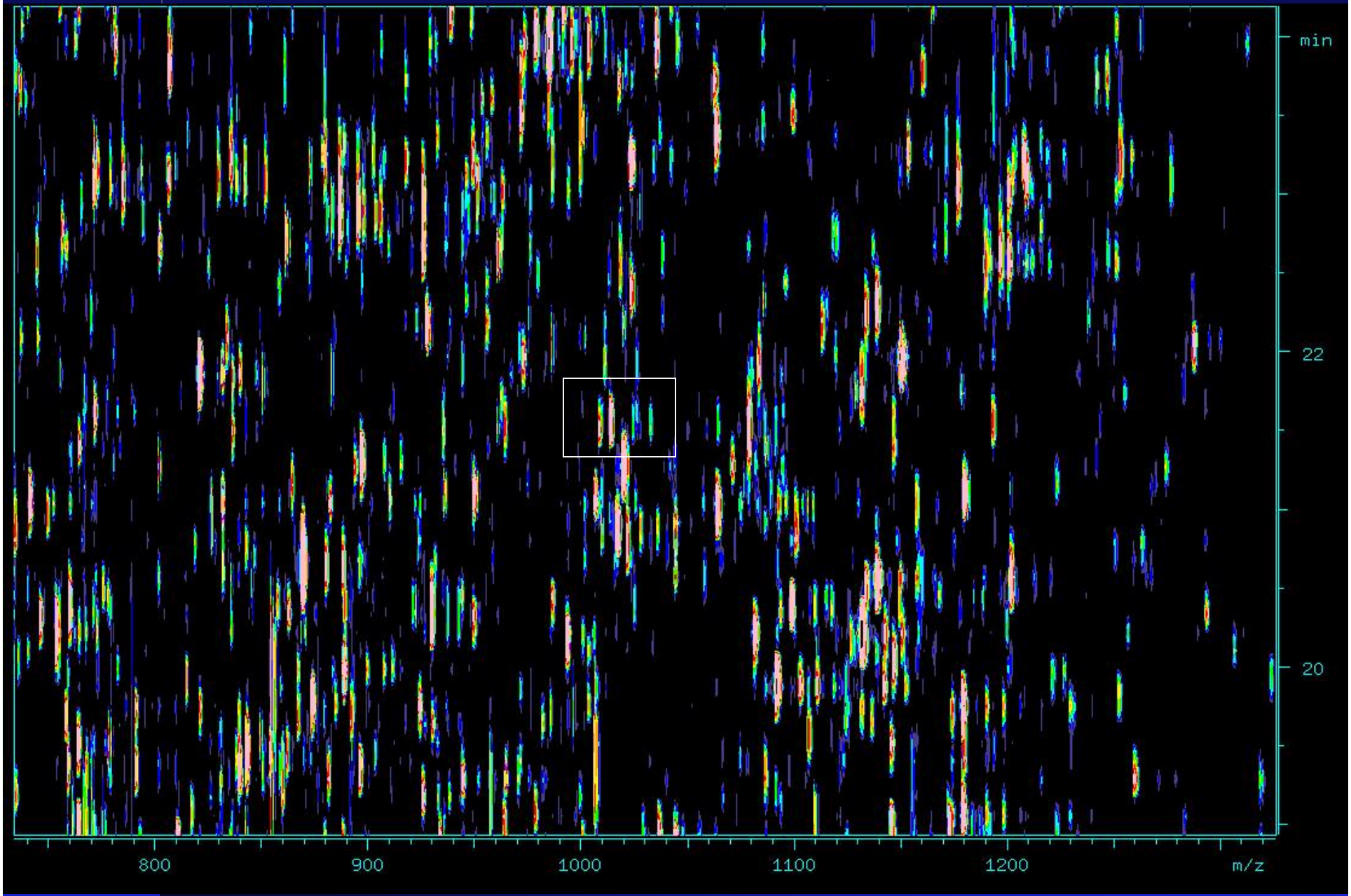
Relative Quantitation

Neat picture Here

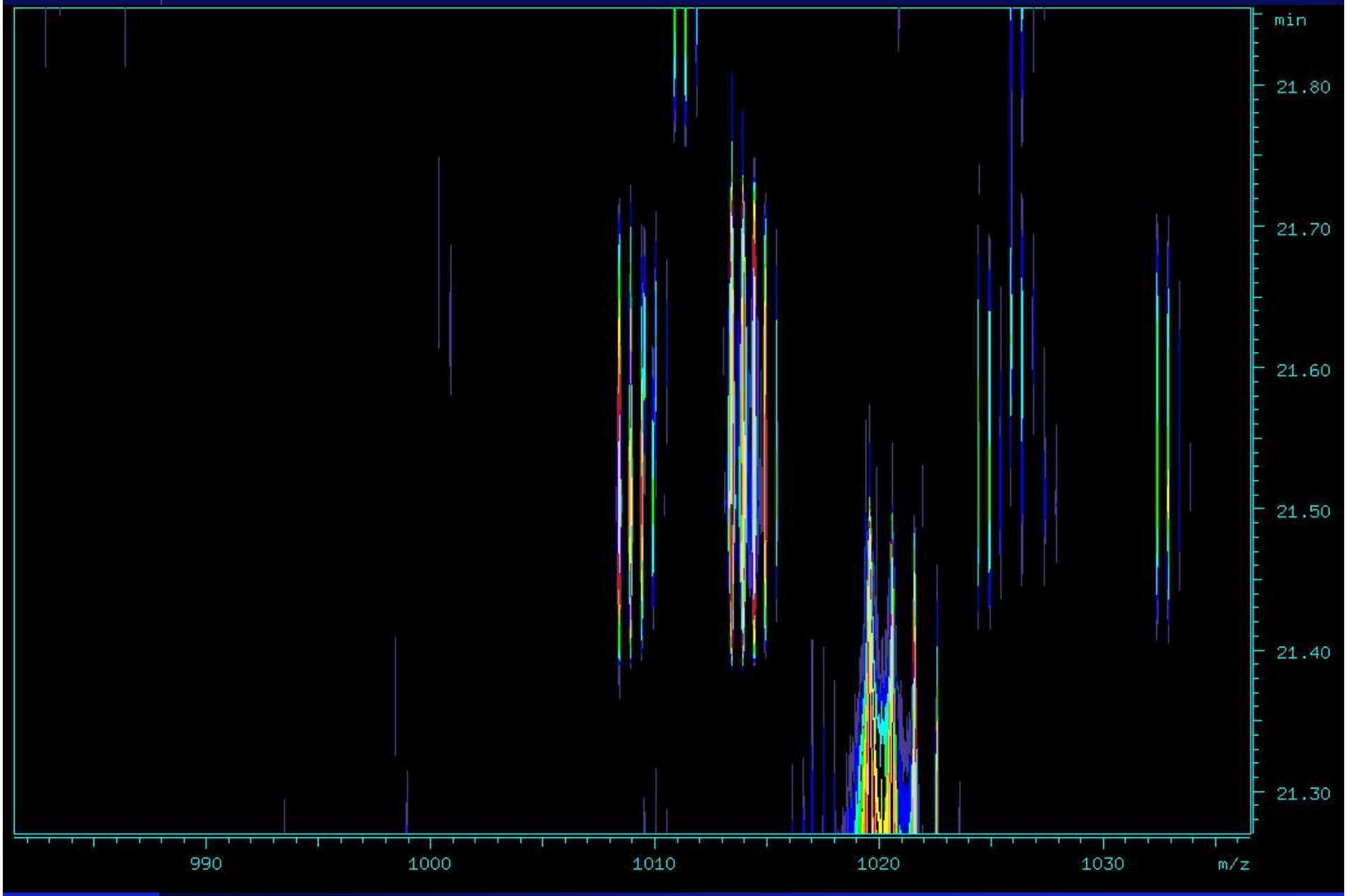
nLC-FTICR MS Relative Quantitation of Oxidized Sites



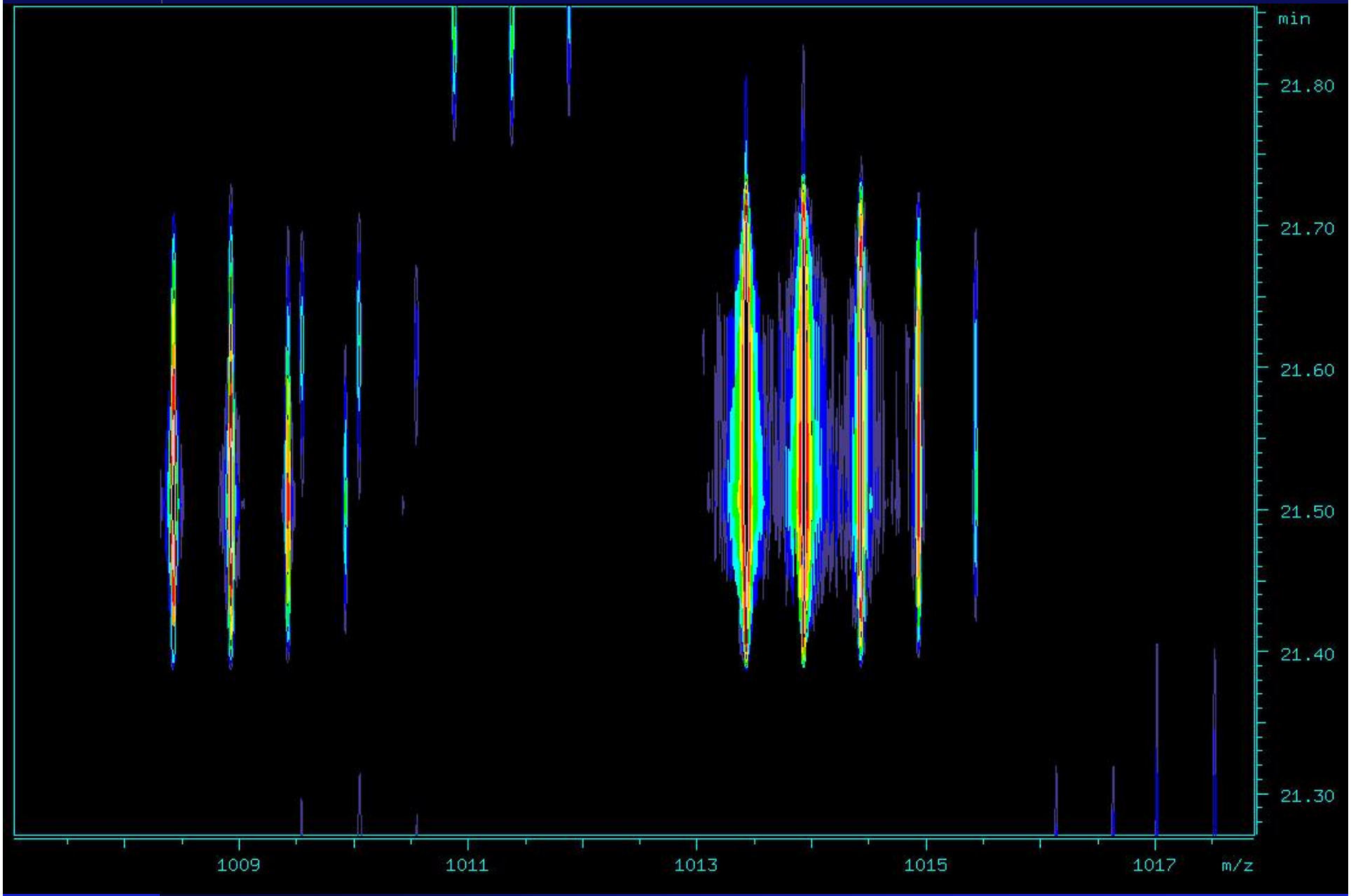
nLC-FTICR MS Relative Quantitation of Oxidized Sites



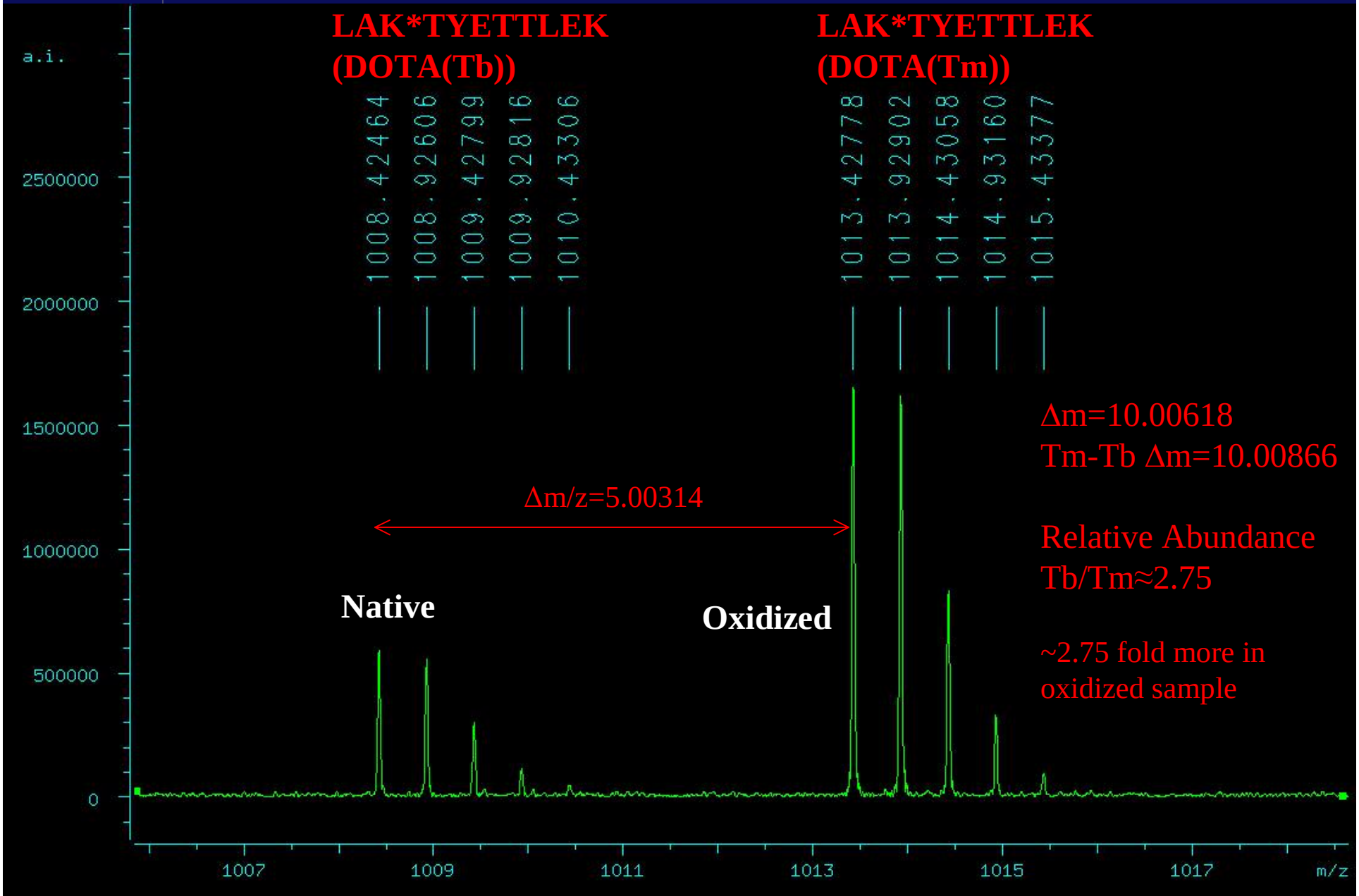
nLC-FTICR MS Relative Quantitation of Oxidized Sites



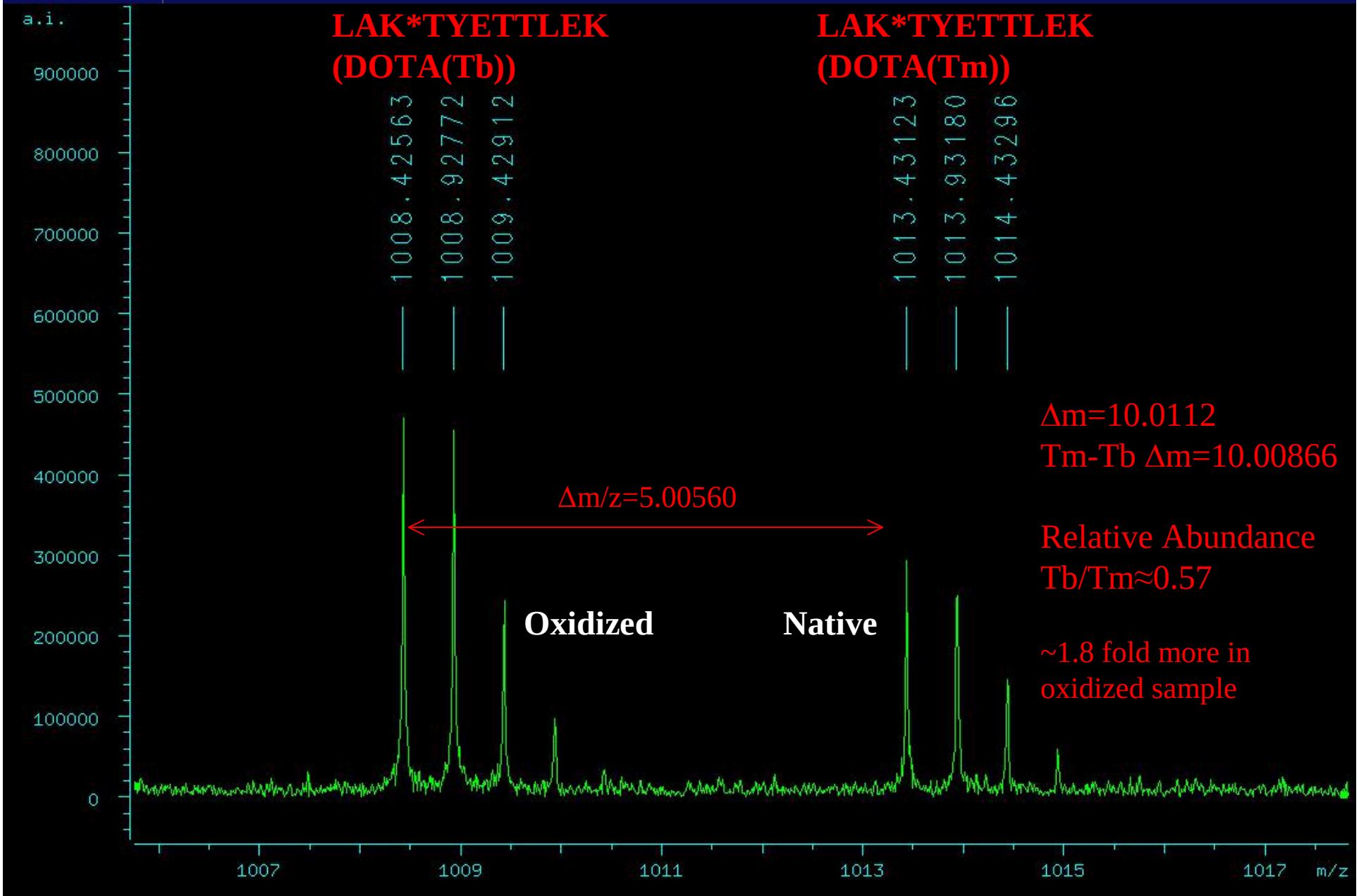
nLC-FTICR MS Relative Quantitation of Oxidized Sites



nLC-FTICR MS Relative Quantitation of Oxidized Sites



nLC-FTICR MS Relative Quantitation of Oxidized Sites



Δm=10.0112
Tm-Tb Δm=10.00866

Relative Abundance
Tb/Tm≈0.57

~1.8 fold more in oxidized sample

Quantitation Summary

- Relative quantitation achieved
- Validated with reverse tagging experiment
(Some experimental error observed)
- Oxidation site preference found
(Potential for site specific biomarkers validated)

	Native Tb/Oxidized Tm	Oxidized Tm /Native Tb	Average
LD*E*LRD*E*GK	2.5	3.2	2.9
TPVSDR*VTK	11.5	8.0	9.8
LDELRDEGK*ASSAK	1.6	3.5	2.6
LK*ECCEK*PLLEK	1.6	2.5	2.1
LAKTYETTLEK	2.8	1.7	2.3
YIC†ENQDSISSK*LK	5.3	5.7	5.5

HSA Oxidation: Conclusion

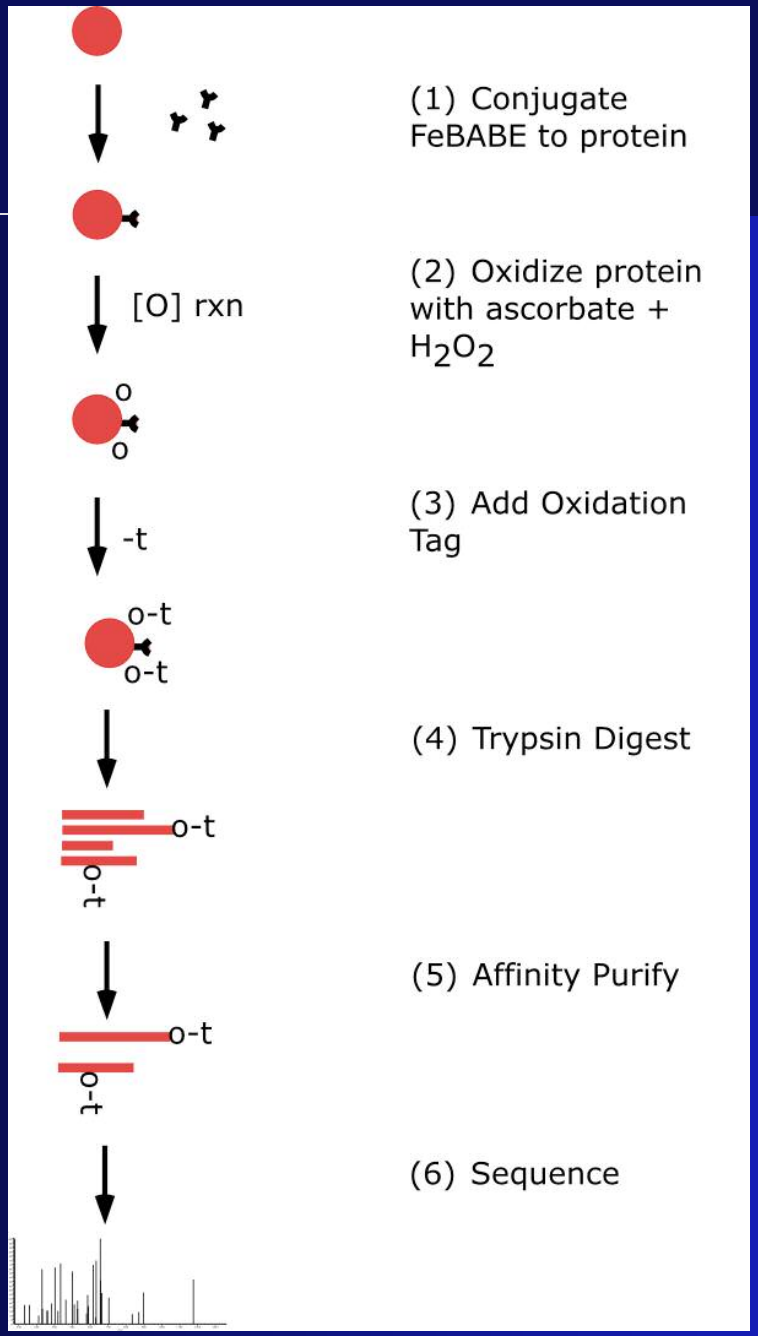
- All oxidized peptides mapped to protein surface (potential low resolution surface mapping technique ala H-D exchange)
- Knowledge of oxidized peptide not necessary for detection (can be understood after the fact)

- Use of tag specific antibodies enables low level detection of oxidized proteins in complex matrices such as serum.
- Relative quantitation achieved

Future Work

- Multiplexed quantitation of clinical samples
- Biomarker discovery using relative quantitation
- Intermolecular interactions (Protein-Protein)

Scheme to Extract Spatial Information Using FeBABE and Lanthanide DOTA Tags



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